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THE THERAPEUTIC EFFICACY OF PHENYL ARSENOXIDES IN MOUSE AND RABBIT TRYPANOSOMIASIS (*TRYP. EQUIPERDUM*) [1]

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A series of mono- and di-substituted phenyl arsenoxides, embracing a wide variety of substituent groups, has been prepared in this laboratory (*1*). Previous communications have dealt with their spirocheticidal activity in relation to their possible usefulness in the treatment of syphilis (*2*). The present paper describes their trypanocidal activity (*Tryp. equiperdum*) in vitro and in vivo, the latter in comparison with representative arsonic acids.

I. Methods and Materials

A. TRYPANOCIDAL ACTION IN VITRO

Rats and white mice were inoculated intraperitoneally by the injection of 1 cc. and 0.1 cc., respectively, of a trypanosome suspension containing 10^7 organisms per cc. The animals were bled from the heart into 5 percent potassium oxalate 48 to 72 hours after inoculation, at the height of the blood infestation. The oxalated blood was rapidly chilled to 0° C. by placing in a freezing cabinet at −25° C. for 5 to 10 minutes, and a rabbit antiserum to rat and mouse red blood cells was then added (0.05 cc. per cc. blood). After thorough admixture, the blood was replaced in the freezing cabinet for a few minutes to allow agglutination of the red blood cells and was then centrifuged, slowly at first to permit the sedimentation of the agglutinated clumps of red blood cells, and then at gradually increasing speed. The red blood cells formed a coherent clump at the bottom of the tube, with a clearly demarcated supernatant white layer of trypanosomes, varying in thickness according to the trypanosome content of the blood. With due care to keep the mixture cold, the rat or mouse antiserum did not cause hemolysis despite the massive

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agglutination of the red blood cells. The oxalated plasma was removed with a capillary pipette and discarded. The layer of trypanosomes was then gently broken up and resuspended in a serum-buffer mixture, care being taken not to disturb the underlying layer of agglutinated red blood cells. The serum-buffer mixture consisted of 1 part of fresh rabbit serum, 2 parts of an isotonic phosphate buffer at pH 7.4, 2 parts of 0.85 percent NaCl, and 1/50 part of 5 percent glucose. In this medium more than 95 percent of the trypanosomes remained fully active for at least 6 hours at room temperature. The final suspension of trypanosomes contained approximately 20 million organisms per cc. and was filtered through No. 12 Whatman filter paper to remove clumps of organisms or minute clumps of red blood cells inadvertently included.

The method used for the assay of trypanocidal action in vitro resembled that used by Yorke and Murgatroyd (*3*), except that the proportion of motile organisms was used as the end point rather than their absolute number, and the incubation time was 2 to 4 hours instead of 24. In each series of assays, the amount of arsenical which immobilized half of the organisms was determined, as compared with a standard reference compound tested at the same time and with the same trypanosomal suspension. Unsubstituted phenyl arsenoxide was used throughout as the reference compound. Minor variations in trypanosome count, in temperature, or in the incubation time did not affect the assay, since the reference compound was tested under the same conditions. A similar technique has been described by one of us for the determination of spirocheticidal activity in vitro (*4*).

TABLE 1.—*Illustrating the method used for the determination of relative trypanocidal action in vitro*

Compound (RC_6H_4AsO or $R_1R_2C_6H_3AsO$)	Dilution used [1]	Cc. of arsenical solution in a total volume of 0.4 cc. (0.4 cc. of trypanosomal suspension then added)							Amount of solution at which 45 percent of organisms remained motile [2]	Relative trypanocidal activity per gram referred to that of phenyl arsenoxide as 100 [3]
		0.4	0.28	0.2	0.14	0.1	0.07	0.05		
		Proportion of motile organisms after 135 minutes at room temperature (23° C.)								
Unsubstituted phenyl arsenoxide (reference compound)	1:4,000,000	----	0	30	>90	----	----	----	0.185	100
p-$(CH_2)_3COOH$	1:1,000,000	0	0	0	36	84	----	----	.13	35
3-NH_2-4-COOH	1:100,000	----	----	0	30	90	----	----	.13	35
p-NH_2	1:2,000,000	----	0	85	----	----	----	----	.24	38
p-$CONHC_2H_4OH$	1:500,000	----	----	----	0	64	----	----	.11	20
Unsubstituted phenyl arsenoxide	1:4,000,000	0	0	20	>90	----	----	----	.18	100

[1] Determined by preliminary orienting experiment.
[2] By interpolation.
[3] $\frac{\text{Amount of reference solution}}{\text{Amount of unknown solution}} \times \frac{\text{Concentration of reference solution}}{\text{Concentration of unknown solution}} \times 100$. Thus, for the first compound listed in the table (the p-$(CH_2)_3COOH$ phenyl arsenoxide) the activity relative to that of phenyl arsenoxide was:

$$\frac{0.182}{0.132} \times \frac{1:4,000,000}{1:1,000,000} \times 100 = 1.39 \times \frac{1}{4} \times 100 = 35.$$

As illustrated in table 1, varying amounts of the arsenical to be tested were distributed in a series of tubes, the volume brought to 0.4 cc. with 0.85 percent NaCl, and an equal volume of the trypanosome suspension added. Similar rows were prepared for each of four to six compounds simultaneously tested, allowing a 5-minute interval between the addition of the organisms to succeeding rows. After 120 to 240 minutes at room temperature, the proportion of motile organisms was determined by direct dark-field observation. With practice it was possible to complete a single assay in 5 minutes, so that in each row the trypanosomes would have remained in contact with the arsenical for the same period of time. As indicated in table 1, the volume of arsenical solution necessary to reduce the proportion of motile organisms to just 45 percent was determined by interpolation: and this value, considered in relation to the corresponding amount of the reference compound and the dilutions employed, gave directly the relative trypanocidal action in vitro (last column of table 1).

B. TRYPANOCIDAL ACTION IN VIVO

(*a*) Male white mice weighing 16 to 20 gm. were inoculated by the intraperitoneal injection of 100,000 organisms (0.1 cc. of a suspension containing 10^6 per cc.). Twenty-four hours later the mice were treated by the intraperitoneal injection of an arsenical solution in a volume of 0.25 to 0.8 cc. Survival for more than 30 days was taken as the criterion of cure. Untreated controls died regularly in 3 to 5 days. In inadequately treated animals, death was usually delayed, but only rarely beyond the twentieth day; and at such intermediate dosages, 20 mice which had survived beyond the thirtieth day were found to be noninfectious.

Between 6 and 15 mice were used in each series of doses; and the minimal curative dose ($CD_{>95}$) and the CD_{50} (the dose which cured 50 percent of the mice) were determined by the Reed-Muench method (*5*) as illustrated in table 2.

TABLE 2.—*Method used for evaluation of therapeutic activity of arsenicals in experimental trypanosomiasis (white mice)*

Mice weighing 16 to 20 gm. were inoculated intraperitoneally with 10^6 organisms (*Trypanosoma equiperdum*) and were treated by a single intraperitoneal injection of arsenical 24 hours later. Survival for more than 30 days was taken as the criterion of cure.

Compound used	Mg./kg.	Dead	Survived	Recalculated after Reed and Muench (*5*)			CD_{50}, mg./kg.	MCD (>95 percent cure), mg./kg.
				Dead	Survived	Cured (percent)		
p-$CONHC_2H_4OH$-C_6H_4AsO	8	0	12	0	28	100	4.1	8.0±
	6	5	6	5	16	74		
	4	6	6	11	10	48		
	3	7	3	18	4	18		
	2	7	1	25	1	4		
	1.5	3	0	28	0	0		

(*b*) Rabbits. The disease caused by *Tryp. equiperdum* in rabbits is not an acute blood infection as it is in rats and mice but is a more chronic disease involving the tissues and manifested particularly by conjunctivitis, blepharitis, rhinitis, and edema and inflammation of the ears, perineal area, and skin (*6*). The animals were inoculated by the intravenous injection of 1 cc. of a suspension containing 10^7 organisms per cc. Fourteen to 17 days later, when the disease had become manifest,[3] they were treated by intravenous injections of the arsenical, repeated once daily for a total of 4 days. Survival for a period of 90 to 140 days after inoculation, with no demonstrable residual involvement, was taken as the criterion of cure. More than 95 percent of the controls died within 12 to 55 days after inoculation, and only 2 of 62 untreated controls survived for more than 100 days. Death was often delayed in inadequately treated animals, most of which died 3 to 55 days after treatment. The minimal curative dose and the CD_{50} were determined from the experimental data as illustrated in table 3.

TABLE 3.—*Method used for evaluation of therapeutic activity of arsenicals in experimental trypanosomiasis (rabbits)*

Rabbits were inoculated by the intravenous injection of 10^7 organisms. Fourteen to 17 days later, after the animal was obviously infected, it was treated by intravenous injections of the arsenical, repeated daily for 4 successive days. Survival for more than 90 to 140 days after inoculation, with no residual evidences of infection, was taken as the criterion of cure. Controls died regularly within 55 days.

Compound used	Mg./kg. per injection	Dead	Survived	Recalculated after Reed and Muench (*5*)			CD_{50}, mg./kg. total	MCD (>95 percent cure), mg./kg. total
				Dead	Survived	Survived (percent)		
p-$(CH_2)_4COOH$ C_6H_4AsO	2	2	5	2	19	90	0.94×4=3.8	2×4=8±
	1.5	2	5	4	14	78		
	1.0	3	4	7	9	57		
	.75	4	3	11	5	31		
	.5	5	2	16	2	11		

C. TOXICITY IN WHITE MICE AND RABBITS

The maximal tolerated dose ($LD_{<5}$), the LD_{50} and the minimal lethal dose ($LD_{>95}$) after a single intraperitoneal injection were determined in white mice as previously described (*4*). The same values were determined in rabbits for a single intravenous injection, and for intravenous injections repeated daily for 4 days.

II. Experimental Results

A. TRYPANOCIDAL ACTION IN VITRO

The relative trypanocidal activities in vitro of the phenyl arsenoxides studied in this respect are summarized in the second vertical

[3] Of 568 animals inoculated, 64 died before treatment. Twelve of these were apparently adventitious deaths, occurring within 1 week; the remaining 52 were probably deaths due to the disease and are not included in the experimental protocols.

column of table 4. As in the case of spirochetes, the unsubstituted phenyl arsenoxide was one of the most active compounds in the series, and substitution in the phenyl ring usually served only to reduce that activity to varying degree. In general, the methyl, chloro, and nitro groups had little or no effect either on trypanocidal activity or toxicity; while amino- and hydroxyl-, acetamido-, amide-, and acid-substituted compounds had decreasing activity, in approximately that order. The one exception encountered to the marked inhibiting effect of acidic substitution on trypanocidal activity was provided by the p-$(CH_2)_3COOH$ compound, which was twelve times more active than any other acid-substituted compound tested (cf. p. 779).

In the evaluation of arsenicals with respect to antisyphilitic activity, the trypanocidal action in mice or rats has often been used as a screening procedure (cf. (*7*)), this despite the finding by several workers (*8*, *9*, *10*) that there is no regular or necessary correlation between trypanocidal and treponemicidal activity. With the present series of phenyl arsenoxides, there was in general a rough qualitative agreement between spirocheticidal and trypanocidal activity in vitro, as seen in table 4 and figure 1. However, with those substituents which had a marked effect in lowering activity, toxicity, or both, the results with the two types of assay often differed widely and were sometimes wholly discrepant. This is shown by the increasing scatter in the left-hand portion of figure 1 and is particularly evident from the last column of table 4, in which are listed the ratios of

$$\frac{\text{treponemicidal activity in vitro}}{\text{trypanocidal activity in vitro}}.$$

That ratio would be 1 were there perfect correlation between the two types of parasiticidal action; and the degree to which those ratios deviated from 1, and the irregularity of that deviation, are a measure of the quantitative unreliability of either assay as a measure of the other. Moreover, compounds are occasionally encountered which, like the p-$(CH_2)_3COOH$ phenyl arsenoxide (cf. point in lower right portion of fig. 1), are highly active against trypanosomes and yet have only a negligible treponemicidal action. There is no reason to doubt that the reverse may also occur.

An in vivo comparison of the treponemicidal and trypanocidal action of arsenicals would be further complicated by their varying absorption, excretion, and chemical modification in different animal species. Thus, as shown in table 5, in a small series of amide-substituted phenyl arsenoxides and their derivatives, there was a sevenfold variation in the ratio of $\frac{\text{treponemicidal action in rabbits}}{\text{trypanocidal action in mice}}$, this despite the chemical and pharmacologic similarity of the compounds tested. It seems clear

TABLE 4.—*The direct trypanocidal and treponemicidal activity in vitro of a series of phenyl arsenoxides*

[All values are molar, referred to that of phenyl arsenoxide as 100]

Compound tested (RC_6H_4AsO or $R_1R_2C_6H_3AsO$)		Parasiticidal action in vitro per mole referred to that of phenyl arsenoxide as 100		Ratio of treponemicidal / trypanocidal activities in vitro
		Trypanocidal	Treponemicidal	
Miscellaneous substituents	3-NO_2-4-Cl	106	107	1.0
	p-CH_3	102	102	1.0
	Unsubstituted phenyl arsenoxide	100	100	1.0
	m-Cl	95	110	1.1
	o-Cl	92	83	.9
	o-CH_3	91	84	.9
	p-Cl	90	85	.95
	2,4-diCl	80	100	.8
	3-N=NC_6H_4-4-OH	71	94	1.3
	o-OH	66	84	1.3
	3-NH_2-4-Cl	59	99	1.7
	p-NH_2	57	83	1.5
	2-OH-3-NH_2	41	43	1.05
	p-$NHCOC_6H_4NHCOCH_3$(p′)	40	32	.8
	p-$NHCOCH_3$	35	57	1.6
	p-$CH_2NHCOCH_3$	32	21	.7
	p-$NHSO_2C_6H_4NH_2$(p′)[1]	31	45	1.4
	p-$NHCOCH_2NH_2$	31	24	.8
	3-OH-4-NH_2	30	41	1.3
	3-NH_2-4-OH	27	38	1.4
	3-$NHCOCH_3$-4-OH	3.0	24	8.0
Amides and amide substituents	p-CH=$CHCONH_2$	73	41	.56
	p-$(CH_2)_2CONH_2$	60	33	.55
	3-NH_2-4-$CONH_2$	52	36	.7
	3-OH-4-$CONH_2$	48	45	.9
	p-$CONH_2$	45	45	1.0
	p-$CONHC_2H_4OH$	39	22	.6
	m-$CONH_2$	39	41	1.0
	p-$SO_2N(C_2H_5)_2$	35	101	2.9
	p-$CONHCONH_2$	34	34	1.00
	p-CH_2CONH_2	31	20	.7
	p-$CONHC_6H_4NHCOCH_3$(p′)	29	9	.3
	p-$NHCONH_2$	29	38	1.3
	p-OCH_2CONH_2	26	52	2.0
	p-SO_2NH_2	24	29	1.2
	p-$CONHCH_2CN$	19.5	27.0	1.4
	p-$CONHCH_2CONH_2$	15.0	24	1.6
	p-SO_2NHCH_3	12.6	72	5.7
	p-$SO_2NHC_2H_4OH$	9.0	23	2.6
	m-SO_2NH_2	7.8	21	2.7
	p-$CH_2CONHCH_2CONH_2$	1.5	10.6	7.0
	p-$SO_2NHCH_2CONH_2$	1.4	9.8	7.0
	p-$CONHCH_2COOH$	.22	.7	3.2
Acidic substituents	p-$(CH_2)_3COOH$	54	1.9	.04
	p-$CH(C_2H_5)COOH$	9.9		
	p-$(CH_2)_4COOH$	7.5	22	3.0
	p-CH_2COOH	4.7	4.2	.9
	p-OCH_2COOH	4.5	5.2	1.15
	3-NH_2-4-COOH	4.0	20	5.0
	o-COOH	3.2	28	8.8
	p-$(CH_2)_2COOH$	2.8	4.1	1.5
	p-CH=CHCOOH	2.0	17	8.5
	p-COOH	.45	6.7	15
	p-CHOHCOOH	.3±	2.8	9
	p-SO_3H	.06	3.4	57

[1] Obtained from the laboratories of the Squibb Institute for Medical Research.

that neither experimental infection can be safely substituted for the other in the evaluation of therapeutic activity.

Most phenyl arsenoxides are active as such, and not by virtue of their conversion to other compounds in vivo. One would therefore anticipate a fairly good correlation between their trypanocidal activity

in vitro and therapeutic action in vivo. Yorke, Murgatroyd, and Hawking (*11*) found such a correlation in the trivalent arsenic compounds studied by them. In the present series of compounds, the trypanocidal activity in vitro has been so closely correlated with therapeutic action in vivo as to constitute a reliable screening pro-

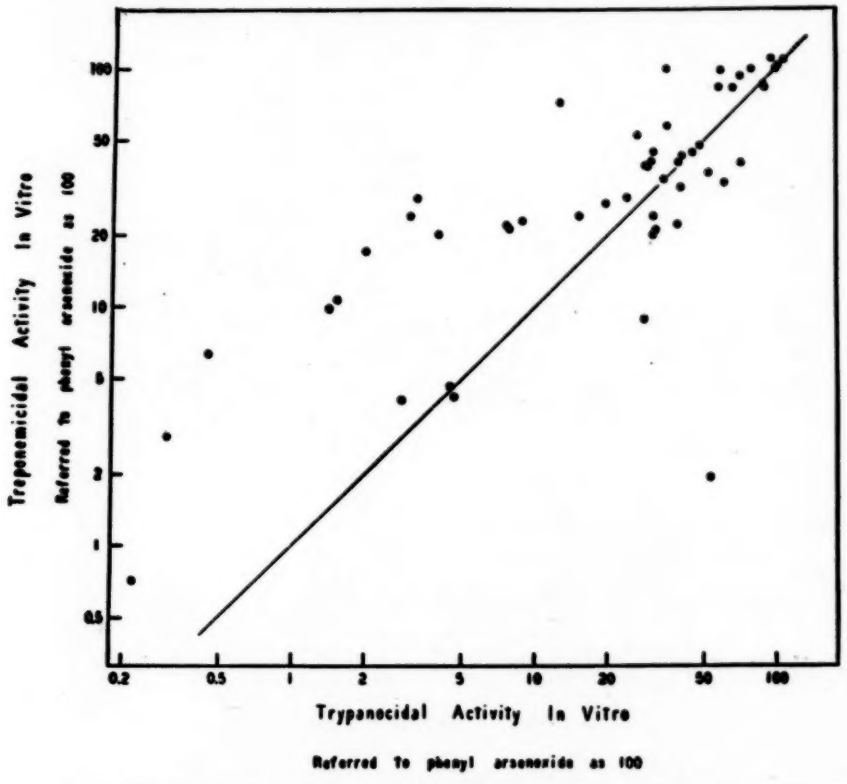

FIGURE 1.—The correlation between the trypanocidal and treponemicidal activity of phenyl arsenoxides in vitro.

TABLE 5.—*Showing the lack of correlation between the therapeutic activity of phenyl arsenoxides in mouse trypanosomiasis and rabbit syphilis*

Compound ($R_1C_6H_4AsO$ or $R_1R_2C_6H_2AsO$)	CD_{50} dose in mouse trypanosomiasis mg./kg.	CD_{50} dose in rabbit syphilis mg./kg.[1]	Ratio of treponemicidal / trypanocidal activity
3-NH_2-4-OH	1.6	3.0	0.5
3-NH_2-4-$CONH_2$	1.7	3.7	.5
p-$CH=CHCONH_2$	2.3	>2	>1.0
p-OCH_2CONH_2	3.1	4±	.8
p-$CONH_2$	3.5	2.8	1.3
p-$NHCONH_2$	3.8	4.6	.8
p-$CONHC_2H_4OH$	4.1	10	.4
p-$CONHCH_2CONH_2$	5.0	15	.3
p-SO_2NH_2	7.1	6	1.2
p-$SO_2NHC_2H_4OH$	23	11	2.0
p-$SO_2NHCH_2CONH_2$	36	25±	1.4±

[1] After Eagle, Hogan, Doak, and Steinman (*12*).

cedure. As shown in table 6 and figure 2, those compounds which are highly active in vitro were active in the treatment of mouse trypanosomiasis;[4] those ineffective in vitro proved relatively inactive in vivo; and as in the case of treponemicidal action (*12*) there was a satisfactory correlation between the two.

TABLE 6.—*The relative trypanocidal activity in vitro and in vivo of a series of phenyl arsenoxides*

	Compound tested (RC_6H_4AsO or $R_1R_2C_6H_4AsO$)	Relative trypanocidal action in vitro (molar) referred to that of—		Trypanocidal action in vivo		Ratio of activities in vivo in vitro
		Unsubstituted phenyl arsenoxide as 100 (from table 4)	p-$CONH_2$ phenyl arsenoxide as 100	CD_{50} mg./kg.	Molar activity referred to p-$CONH_2$ phenyl arsenoxide as 100 [1]	
Miscellaneous substituents.	Unsubstituted phenyl arsenoxide	100	222	>3		
	p-NH_2	57	127	>4	<100	
	2-OH-3-NH_2	41	91	2.9	104	1.1
	p-$NHCOC_6H_4NHCOCH_3$	40	89	4.2	125	1.4
	p-$CH_2NHCOCH_3$	32	71	3.3	100	1.4
	3-NH_2-4-OH	27	60	1.6	246	4.1
	3-$NHCOCH_3$-4-OH	3.0	6.7	7.1	60	9.0
Amides and amide derivatives.	p-$CH=CHCONH_2$	73	162	2.3	158	1.0
	p-$(CH_2)_3CONH_2$	60	133	2.4	165	1.3
	3-NH_2-4-$CONH_2$	52	115	1.66	210	1.1
	3-OH-4-$CONH_2$	48	106	1.7	223	2.1
	p-$CONH_2$	45	100	3.3	100	1.00
	p-$CONHC_2H_4OH$	39	87	4.1	95	1.1
	p-$CONHCONH_2$	34	75	4.5	92	1.2
	p-$CONHC_6H_4NHCOCH_3$(p′)	29	65	6.2	85	1.3
	p-$NHCONH_2$	29	65	3.8	99	1.5
	p-OCH_2CONH_2	26	58	3.1	128	2.2
	p-SO_2NH_2	24	53	7.1	56	1.1
	p-$CONHCH_2CONH_2$	15	33	5.0	81	2.5
	p-$SO_2NHC_2H_4OH$	9.0	20	23	20	1.0
	p-$CH_2CONHCH_2CONH_2$	1.5	3.3	15	33	10.0
	p-$SO_2NHCH_2CONH_2$	1.4	3.1	35.6	13	4.2
	p-$CONHCH_2COOH$	.22	.5	>24	<18	
Acidic substituents.	p-$(CH_2)_3COOH$	54	113	1.6	242	2.0
	p-CH_2COOH	4.7	10.4	7.3	48	4.6
	p-OCH_2COOH	4.5	10	7.7	48	4.8
	p-$(CH_2)_3COOH$	2.8	6.2	11	34	5.5
	p-SO_3H	.06	.1	>32	<15	

[1] This compound arbitrarily used as reference base instead of the unsubstituted phenyl arsenoxide. The latter was not curative even in sublethal doses.

Despite the good qualitative agreement between trypanocidal action in vitro and in vivo, it is to be noted that weakly active compounds were regularly more effective in vivo than might have been anticipated from their trypanocidal activity. This is shown in the last column of table 6 and in figure 3, in which trypanocidal action in vitro is plotted against the ratio of $\frac{\text{therapeutic activity in vivo}}{\text{trypanocidal action in vitro}}$ activities. The less active the compound in vitro (left side of figure), the higher was that ratio. This puzzling observation may be related

[4] It is to be noted in table 5 and figure 2 that the unsubstituted phenyl arsenoxide could not be used as the reference compound in the in vivo assays, since it failed to cure even in sublethal doses.

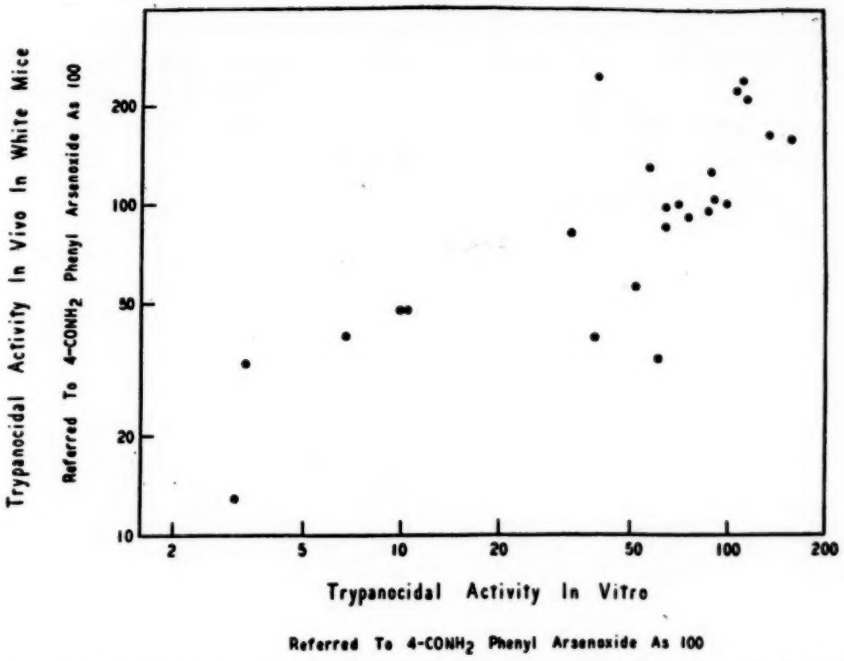

FIGURE 2.—The correlation between the trypanocidal activity of phenyl arsenoxides in vitro and in vivo.

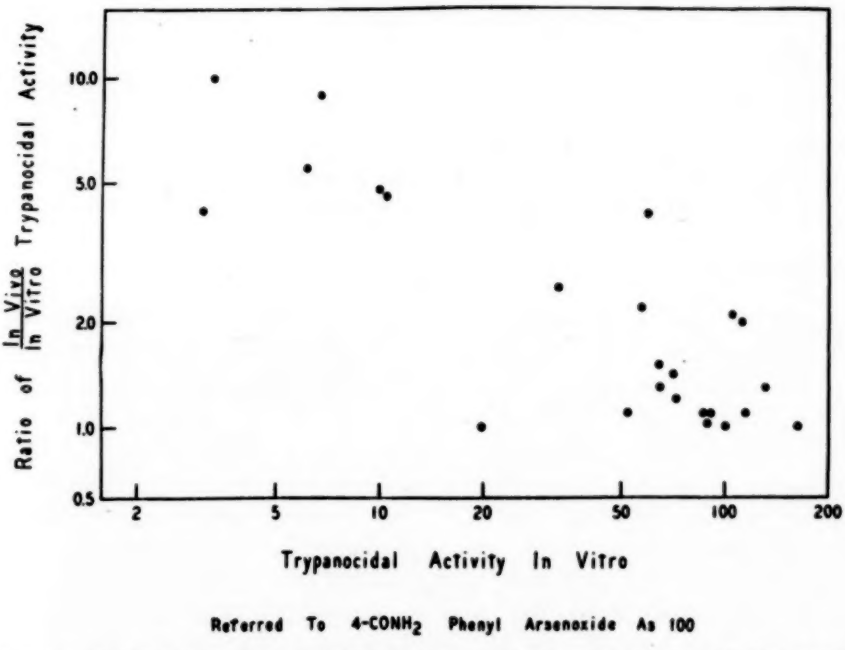

FIGURE 3.—Showing that weakly trypanocidal phenyl arsenoxides are more effective in vivo than would be suggested by their direct trypanocidal activity.

to the fact that most of these compounds are relatively nontoxic and are therefore not bound by the tissues as rapidly or as completely as the more toxic derivatives of phenyl arsenoxide (*14*). Under such circumstances they may remain free in the tissue fluids at higher concentrations and for longer periods and thus exert a trypanocidal effect considerably in excess of that anticipated on the basis of their intrinsic trypanocidal activity. Nevertheless, if due cognizance is taken of this factor, the trypanocidal activity of phenyl arsenoxides in vitro apparently offers a helpful orientation to their therapeutic activity in vivo.

B. THERAPEUTIC ACTIVITY AND CHEMOTHERAPEUTIC INDEX IN MICE AND RABBITS

Twenty-nine of the present series of compounds were tested in the treatment of experimental trypanosomiasis in white mice. Their toxicity, therapeutic activity, and chemotherapeutic index, expressed both as $\frac{\text{maximal tolerated dose}}{\text{minimal curative dose}}$ and $\frac{LD_{50}}{CD_{50}}$, are given in table 7, in which the compounds are arranged in the order of decreasing chemotherapeutic index. For comparison, the corresponding values for a series of arsonic acids are given in table 8. As previous workers have found, the phenyl arsenoxides were regularly far more effective than the corresponding arsonic acids, only 1/60 to 1/300 as much being required to effect cure. Because of that greater activity, and despite their higher toxicity, the arsenoxides regularly gave a more favorable margin between the curative and toxic levels, exceeding two- to sevenfold that provided by the corresponding arsonic acids. This is contrary to the findings of Gough and King (*13*) who, comparing the chemotherapeutic index $\left(\frac{\text{maximum tolerated dose}}{\text{minimum curative dose}}\right)$ of phenyl arsonic acids and the corresponding arsenoxides in the treatment of mouse trypanosomiasis (*Tryp. equiperdum*), found no significant difference in the case of the p-$CONH_2$ and p-SO_2NH_2 compounds and some of their derivatives.

The four phenyl arsenoxides in our series which gave the best indices in mouse trypanosomiasis, and two phenyl arsonic acids, were also tested in the treatment of rabbit trypanosomiasis. The latter chronic tissue disease was usually more difficult to cure than the acute blood infection of mice (compare col. 4 of table 7 and col. 5 of table 9). In addition, the arsenic compounds were several times more toxic in rabbits.[5] In consequence, the chemotherapeutic indices of both pentavalent and trivalent arsenicals in rabbit trypano-

[5] The apparently higher LD_{50} values of table 9 represent the *total* amount administered in four daily injections.

TABLE 7.—*The toxicity, therapeutic efficacy and chemotherapeutic index of phenyl arsenoxides in the treatment of mouse trypanosomiasis* (Tryp. equiperdum)

Phenyl arsenoxides tested (RC_6H_4AsO or $R_1R_2C_6H_3AsO$)	Toxicity		Therapeutic activity		Chemotherapeutic index	
	LD_{50} [1] mg./kg.	MTD [1] mg./kg.	CD_{50} [1] mg./kg.	MCD [1] mg./kg.	$\frac{LD_{50}}{CD_{50}}$	$\frac{MTD}{MCD}$
3-NH_2-4-OH ("mapharsen")	42.6	33.6	1.6	3.4	26.6	9.9
3-NH_2-4-$CONH_2$	47	39	1.7	4.3	28.3	9.0
p-$(CH_2)_3COOH$	33.4	26	1.6	3.4	20.5	7.6
p-$CONHCH_2CONH_2$	80	64	5.0	9.5	16.0	6.7
p-$CONHC_2H_4OH$	64	51	4.1	7.6	15.7	6.7
p-$CONHCONH_2$	48.4	40	4.5	7.7	10.8	5.2
p-$CH_2NHCOCH_3$	38	29	3.3	6.2	11.5	4.7
p-$NHCOC_6H_4NHCOCH_3$	59	41	4.2	10±	14.1	4.1±
p-SO_2NH_2	63	42	7.1	11.3	8.9	3.7
p-$(CH_2)_3CONH_2$	21.6	15.0	2.4	4.2	9.0	3.5
3-OH-4-$CONH_2$	12.3	9.5±	1.7	2.85	7.2	3.3
p-$CH_2CONHCH_2CONH_2$	109	85±	15.0	26.0	7.2	3.2
p-$CH=CHCONH_2$	28	20	2.3	6.8	12.2	3.0
p-$NHCONH_2$	35	24	3.8	8.0	9.2	3.0
p-OCH_2CONH_2	33	17	3.1	6.6	10.7	2.6
p-$SO_2NHC_2H_4OH$	84	71	23.0	33.0	3.6	2.1
p-$CONH_2$	27.5	17	3.5	8.6	7.9	2.0
p-$(CH_2)_3COOH$	38.8	30	11.0	16.7	3.5	1.8
3-$NHCOCH_3$-4-OH	25.5	18.1	7.1	13.0	3.5	1.4
p-$SO_2NHCH_2CONH_2$	100	64	36.0	52.0	2.8	1.2
p-OCH_2COOH	11.1	8.2	7.7	14.0	1.5	0.6
Unsubstituted phenyl arsenoxide	3	1.5	>3.0	>3.0	<1.0	<1.0
p-$CONHCH_2COOH$	21.2	14	>24.0	>24.0	<0.9	<0.8
p-CH_2COOH	6.4	4.3	7.3	>8.0	0.9	<0.5
p-SO_3Na	13	9.4	>32.0	>32.0	<0.4	<0.3

[1] LD_{50}=dose which killed half of animals.
MTD=maximal tolerated dose (<5 percent killed).
CD_{50}=dose which cured half of animals.
MCD=minimal curative dose (>95 percent cured).

TABLE 8.—*The toxicity, therapeutic efficacy, and chemotherapeutic index of phenylarsonic acids in the treatment of mouse trypanosomiasis* (Tryp. equiperdum)

Phenylarsonic acids tested ($R_1C_6H_4AsO_3H_2$ or $R_1R_2C_6H_3AsO_3H_2$)	Toxicity		Therapeutic activity		Chemotherapeutic index		Chemotherapeutic index of corresponding phenyl arsenoxide (from table 7)	
	LD_{50} [1] mg./kg.	MTD [1] mg./kg.	CS_{50} [1] mg./kg.	MCD [1] mg./kg.	$\frac{LD_{50}}{CD_{50}}$	$\frac{MTD}{MCD}$	$\frac{LD_{50}}{CD_{50}}$	$\frac{MTD}{MCD}$
3-NH_2-4-OH-	2,400	1,300	260	380	9	3.4	26.6	9.9
p-OCH_2CONH_2-	2,000±	1,200±	350	420	5.7	3.0	10.7	2.6
p-$CONHCH_2CONH_2$-	7,000±	5,400±	1,500	2,800	5±	2±	16.0	6.7
p-OH-	1,600	900	300	600	5.3	1.5	-------	-------
p-$NHCH_2CONH_2$- (tryparsamide)	3,750	2,500	950	1,550	4.0	1.6	-------	-------
p-NH_2-	480	300	120	200	4.0	1.5	-------	-------
p-$(CH_2)_3COOH$-	345	220	94	200±	3.7	1±	20.7	7.6
p-SO_2NH_2-	2,200	1,400	634	1,200	3.5	1.2	8.9	3.7
3-$NHCOCH_3$-4-OH- ("Stovorsal")	2,800	1,500	2,000	2,400	1.4	<1	3.5	1.4
Unsubstituted phenylarsonic acid	42	30	>40	>40	<1	<1	<1	<1

[1] LD_{50}=dose which killed 50 percent of the animals in 4 days.
MTD=maximal tolerated dose (<5 percent mortality).
CD_{50}=dose which cured 50 percent of the animals (30-day observation).
MCD=minimal curative dose (>95 percent cure).

somiasis were usually less favorable than in the mouse infection. The trivalent phenyl arsenoxides again proved many times more effective than the arsonic acids, only 4 to 16 mg. per kg. of the former being required to cure 50 percent of the animals, as compared with

approximately 600 mg. per kg. of the two arsonic acids. However, because of the higher toxicity of the arsenoxides, their chemotherapeutic indices in rabbits were only slightly greater than those of the arsonic acids. Of the six selected compounds tested in rabbits, the best index was given by the p-$(CH_2)_3COOH$ phenyl arsenoxide, with an $\frac{LD_{50}}{CD_{50}}$ ratio of 4.4 as compared with one of 2.8 tryparsamide.

TABLE 9.—*The toxicity, therapeutic efficacy and chemotherapeutic index of arsenicals in rabbit trypanosomiasis*

[Four successive daily intravenous injections]

Type of compound tested	Substituents	Toxicity [1]		Therapeutic efficacy [1] CD_{50} mg./kg.	Chemotherapeutic index in rabbits $\frac{LD_{50}}{CD_{50}}$	Chemotherapeutic index in mice $\frac{LD_{50}}{CD_{50}}$ [2]
		LD_{50} mg./kg.	MTD mg./kg.			
Phenyl arsenoxides	p-$(CH_2)_3COOH$	16	8	3.6	4.4	20.5
	3-NH_2-4-OH (mapharsen)	42	31	12±	3.5	26.6
	3-NH_2-4-$CONH_2$	38	25	16±	2.4	28.3
	p-$CONHCH_2CONH_2$	64	48	30.0	2.1	16.0
Phenylarsonic acids	3-NH_2-4-OH	400	250	600±	<1	9.0
	p-$NHCH_2CONH_2$ (tryparsamide)	1,800	1,200	640	2.8	4.0

[1] The values listed in these columns are the total dosages given over the 4-day period.
[2] From table 7.

C. CORRELATIONS BETWEEN CHEMICAL STRUCTURE AND CHEMOTHERAPEUTIC ACTIVITY

The unsubstituted phenyl arsenoxide was one of the most actively trypanocidal and toxic compounds in the present series. Substituent groups usually served only to decrease those two properties, and to varying degrees (table 10). In general, substitution with NO_2, Cl, or CH_3 groups had no significant effect on either trypanocidal activity in vitro or, as previously reported (*2*), on toxicity. Similarly, although the compounds with a single -OH or -NH_2 group were somewhat less toxic than the parent compound, they were usually correspondingly less active. These compounds, like the parent phenyl arsenoxide, would therefore be of no value in the treatment of trypanosomiasis.

There follows in table 10 an intermediate series of compounds in which the substituent group reduced toxicity to a greater degree than it did trypanocidal activity, with the result that the compound had a favorable chemotherapeutic index. Those compounds fell into three general classes: compounds with terminal acetamide groups, aminophenols, and amide- substituted compounds and their derivatives.

All but one of the five acetamido-compounds studied had favorable $\frac{\text{activity}}{\text{toxicity}}$ ratios, due in large measure to the detoxifying effect of the

TABLE 10.—*Correlations between the chemical structure of phenyl arsenoxides and their chemotherapeutic activity* (Tryp. equiperdum)

General type of substituent group	Specific compound	Molar trypanocidal action in vitro, referred to phenyl arsenoxide as 100	Molar toxicity in white mice, referred to phenyl arsenoxide as 100	Ratio of trypanocidal activity in vitro: toxicity, referred to phenyl arsenoxide as 1	Chemotherapeutic index $\left(\frac{LD_{50}}{CD_{50}}\right)$ in mouse trypanosomiasis
Unsubstituted phenyl arsenoxide (reference compound).		100	100	1	No cures in sublethal doses.
NO_2, Cl, CH_3 ("Indifferent" substituents).	3-NO_2-4-Cl	106	100	1.06	
	p-CH_3	102	118	.85	
	m-Cl	95	110	.86	
	o-Cl	92	77	1.2	
	o-CH_3	91	88	1.04	
	p-Cl	90	98	.92	
	2, 4-diCl	80	100	.8	
Terminal -NH_2 and -OH	m-N=NC_6H_3OH(p′)	71	60	1.2	
	o-OH	66	85	.8	
	3-NH_2-4-Cl	59	94	.6	
	p-NH_2	57	57	1.0	
	p-$NHSO_2C_6H_4NH_2$(p′)	31	14	2.2	
	p-$NHCOCH_2NH_2$	31	13.5	2.3	
Terminal acetamide group	p-$NHCOC_6H_4NHCOCH_3$(p′)	40	6.7	6.0	14
	p-$NHCOCH_3$	35	20.5	1.7	
	p-$CH_2NHCOCH_3$	32	7.3	4.4	11.5
	p-$CONHC_6H_4NHCOCH_3$(p′)	12.3	2±	6±	
	3-$NHCOCH_3$-4-OH	3.0	12.4	.25	3.5
Aminophenols	2-OH-3-NH_2	41	79	.5	
	3-OH-4-NH_2	30	10.5	3.0	
	3-NH_2-4-OH	27	6.9	4.0	26.6
Terminal amides	p-CH=$CHCONH_2$	73	9.8	7.4	12.2
	p-$(CH_2)_3CONH_2$	60	13.5	4.4	9.0
	3-NH_2-4-$CONH_2$	52	5.6	9.1	28.3
	3-OH-4-$CONH_2$	48	23	2.1	7.2
	p-$CONH_2$	45	9.6	4.7	7.9
	p-$CONHCONH_2$	34	6.4	5.0	10.8
	p-CH_2CONH_2	31	8.7	3.6	
	p-$NHCONH_2$	29	8.1	3.6	9.8
	p-OCH_2CONH_2	26	9.0	2.9	10.7
	p-SO_2NH_2	24	4.8	5.0	8.9
	p-$CONHCH_2CONH_2$	15	3.9	3.9	16
	m-SO_2NH_2	7.8	6.1	1.3	8.9
	p-$CH_2CONHCH_2CONH_2$	1.5	3.4	.43	7.2
	p-$SO_2NHCH_2CONH_2$	1.4	3.5	.4	2.8
Substituted amides	p-$CONHC_2H_4OH$	39	4.56	8.5	15.7
	p-$SO_2N(C_2H_5)_2$	35	134	.3	
	p-$CONHC_6H_4NHCOCH_3$	29	2±	15±	
	p-$CONHCH_2CN$	20	4.53	4.4	
	p-SO_2NHCH_3	12.6	17.6	.7	
	p-$SO_2NHC_2H_4OH$	9	4.2	2.2	3.6
	p-$CONHCH_2COOH$	.22	15.6	.01	<1
Acidic groups	p-$(CH_2)_3COOH$	54	8.8	6.1	20.5
	p-$CH(C_2H_5)COOH$	9.9	19.5	.5	
	p-$(CH_2)_3COOH$	7.5	8.1	.9	
	p-CH_2COOH	4.7	41	.11	<.9
	p-OCH_2COOH	4.5	25	.2	1.5
	3-NH_2-4-COOH	4.0	15	.3	
	o-COOH	3.2	27	.1	
	p-$(CH_2)_2COOH$	2.8	7.3	.4	3.5
	p-CH=$CHCOOH$	2.0	9.4	.2	
	p-COOH	.45	41.4	.01	
	p-CH(OH)COOH	.35	7.0	.05	
	p-$CONHCH_2COOH$	.22	15.6	.015	<.9
	p-SO_3H	.06	29	.002	<.4

terminal -NHCOCH$_3$ linkage (cf. (*2*)). The exception was the 3-NHCOCH$_3$-4-OH compound, which was relatively nontoxic but inactive both in vitro and in vivo.

The three aminophenols tested were actively trypanocidal; but the 3-NH$_2$-4-OH compound, because of its paradoxically low toxicity, gave the most favorable $\frac{\text{activity}}{\text{toxicity}}$ ratio. This confirms the results obtained in the assay of treponemicidal activity (*2*). In mouse trypanosomiasis it gave the highest $\frac{LD_{50}}{CD_{50}}$ index of all the phenyl arsenoxides tested. In rabbits, however, this compound was less active and gave a lower chemotherapeutic index than the p-(CH$_2$)$_3$COOH arsenoxide discussed in a following paragraph.

The favorable effect of amide-substitution on the chemotherapeutic activity of phenylarsonic acids and phenyl arsenoxides has been pointed out by Gough and King (*13*). In the present series of phenyl arsenoxides also, amide-substitution regularly resulted in compounds with a favorable index, whether against spirochetes (*2*, *12*) or, as here found, against trypanosomes. This favorable effect was due primarily to the detoxifying effect of the amide-linkage, the molar toxicities of the 14 such compounds in the present series varying between 2.3 and 24 percent that of phenyl arsenoxide. Although their direct trypanocidal activity varied within wide limits, from 0.55 to 73 percent that of the unsubstituted phenyl arsenoxide, the ratio of $\frac{LD_{50}}{CD_{50}}$ varied between 2.8 and 28.3.

The effect of substitution in the amide groups on the chemotherapeutic properties of phenyl arsenoxides has been discussed in detail in a preceding communication in relation to treponemicidal action (*2*, *12*). As there indicated, and confirmed for the few such compounds studied with respect to trypanocidal action, replacement of an amide hydrogen with a -CH$_3$, a -C$_2$H$_5$ group, or with a group containing a terminal -COOH, caused a shift in the properties of the compound toward those characteristic of the new terminal group, with an increase in toxicity and a lower $\frac{\text{activity}}{\text{toxicity}}$ ratio. On the other hand, similar substitution with groups containing a terminal hydroxyl, acetamido, or nitrile linkage usually affected both activity and toxicity to approximately the same degree, so that the favorable influence of the amide group was not adversely affected.

Of the disubstituted compounds, the 3-NO$_2$-4-Cl, 3-NH$_2$-4-Cl, and 2,4-diCl, each with two "indifferent" substituents, had the expected high toxicity, high activity, and an $\frac{\text{activity}}{\text{toxicity}}$ ratio not significantly different from that of the unsubstituted compounds. In the case of

the 3-NH_2-4-$CONH_2$ compound, the detoxifying effect of the benzamide group was enhanced by the adjacent amino group, resulting in a compound with a highly favorable chemotherapeutic index. In the analogous 3-OH-4-$CONH_2$ compound, however, the detoxifying effect of the amide group was impaired by the adjacent hydroxyl group, without a commensurate increase in activity. In both cases the effect of the second group on trypanocidal activity corresponded with that observed in the assay of treponemicidal activity (*12*).

As was true in the case of spirocheticidal action, acidic substituents usually caused a striking decrease in trypanocidal activity. Of the 13 such compounds included in the present series and listed at the bottom of table 10, 12 (the p-SO_3H, o-COOH, p-COOH, p-CH(OH)COOH, p-CH_2COOH, p-$(CH_2)_2COOH$, p-CH=CHCOOH, p-$CH(C_2H_5)COOH$, p-$(CH_2)_5COOH$, p-OCH_2COOH, p-$CONHCH_2COOH$, and 3-NH_2-4-COOH phenyl arsenoxides) had molar trypanocidal activities varying between 0.06 and 9.9 percent that of the parent phenyl arsenoxide. Although their toxicity was also reduced, it was usually not sufficiently low to give the compounds a favorable chemotherapeutic index. Of 5 such compounds studied in mouse trypanosomiasis, 3 failed to cure even at sublethal levels, and in the other 2 the ratios of $\frac{LD_{50}}{CD_{50}}$ were 3.5 and 1.5.

As seen in comparing any acid in table 10 with the corresponding amide, the striking decrease in the trypanocidal activity of phenyl arsenoxide caused by acid substituents was largely counteracted by conversion to the amide. As will be shown in detail in a following paper, the inhibitory effect of acidic groups is related to the failure of the charged anion to combine with the cell and thus exert its parasiticidal action.

The sole exception to the marked inhibitory effect of acidic substitution on trypanocidal action was provided by the p-$(CH_2)_3COOH$ phenyl arsenoxide, which from the chemotherapeutic point of view is the most promising compound so far studied. The experimental data with this compound are summarized in table 11. In vitro, its trypanocidal activity was 54 percent that of the unsubstituted compound. In the treatment of mouse trypanosomiasis, it was one of the most active compounds of the series, a single injection of 1.6 mg. per kg. curing 50 percent, and 3.4 mg. per kg. curing more than 95 percent of the mice. The chemotherapeutic index $\frac{LD_{50}}{CD_{50}}$ in that species was 20.5, the third highest in the entire series of compounds studied. In the treatment of rabbit trypanosomiasis, this compound was the most active of the six selected compounds studied (CD_{50} in four daily injections=3.6 mg. per kg.), and gave the

TABLE 11.—*The trypanocidal activity in vitro and in vivo, toxicity, and chemotherapeutic index of p-arsenoso-phenylbutyric acid, compared with that of tryparsamide*

[All dosages are expressed in mg./kg. In the rabbits, treated by 4 daily injections, the dosages listed are the totals over the 4-day period.]

CD_{50}=dose which cured half of animals.
MCD ("minimum curative dose") = dose which cured >95 percent.
LD_{50}=dose which killed half of animals.
MTD ("maximal tolerated dose")=dose which killed <5 percent.

	White mice (Acute blood infection, treated by single intraperitoneal injection)						Rabbits (Chronic tissue infection, treated by 4 intravenous injections at 24-hour intervals)			
	Therapeutic activity		Toxicity		Chemotherapeutic index		Therapeutic activity	Toxicity		Chemotherapeutic index
	CD_{50}	MCD	LD_{50}	MTD	$\frac{LD_{50}}{CD_{50}}$	$\frac{MTD}{MCD}$	CD_{50}	LD_{50}	MTD	$\frac{LD_{50}}{CD_{50}}$
p-Arsenoso-phenylbutyric acid.	1.6	3.4	33	26	21	7.6	3.6	16	8	4.4
Tryparsamide	940	1,560	3,750	2,500	4.0	1.6	640	1,800	1,200	2.8

most favorable chemotherapeutic index (the ratio of $\frac{LD_{50}}{CD_{50}}$ =4.4). Were it not for its unexpectedly high toxicity in rabbits, exceeding fivefold that observed in mice, the superiority of this compound would have been even more striking. As will be shown in detail in a following paper, it has the further important property of being effective against strains of trypanosomes resistant to most other arsenicals.

No ready explanation can be given for the high activity of this compound. It is to be noted (cf. table 10) that the homologous series consisting of the p-COOH, p-CH_2COOH, p-$(CH_2)_2COOH$, p-$(CH_2)_3COOH$, and p-$(CH_2)_5COOH$ phenyl arsenoxides had molar trypanocidal activities in vitro of 0.45, 4.7, 2.8, 54, and 7.5, respectively. The corresponding values for molar toxicity in mice were 41, 41, 7.3, 8.8, and 8.1. In neither activity nor toxicity is the series progressive, and the high trypanocidal action of the p-$(CH_2)_3COOH$ compound is anomalous.

Whether the high activity of the butyric acid compound extends to species of trypanosomes pathogenic for man, and if so, whether it is effective in the chronic as well as in the early stage of the human infection, are points now being investigated. Its high activity in the experimental rabbit infection, which resembles the chronic disease of man in that the tissues themselves are involved in a chronic inflammatory process, is of promise in this connection.

III. Summary

1. Fifty-four phenyl arsenoxides were assayed with respect to trypanocidal action (*Tryp. equiperdum*) in vitro. The toxicity and

therapeutic efficacy of 26 were studied in white mice, and 4 were further assayed in the treatment of rabbit trypanosomiasis. For comparison, 9 arsonic acids were assayed in mouse trypanosomiasis, and 2 in the rabbit disease.

2. Although there was a rough correlation between the trypanocidal and treponemicidal activity of phenyl arsenoxides, the two assays were sometimes wholly discrepant. There was a sufficiently close correlation between the trypanocidal activity in vitro and in vivo to justify the use of the former as a screening procedure with respect to therapeutic activity. Weakly active compounds were, however, usually more effective in vivo than would have been anticipated from their direct trypanocidal action.

3. Phenyl arsenoxides regularly gave a more favorable chemotherapeutic index in the treatment of mouse trypanosomiasis than the corresponding arsonic acids. The chemotherapeutic index $\left(\frac{LD_{50}}{CD_{50}}\right)$ of nine arsonic acids varied between 1.4 and 9, to be compared with indices of 3.5 to 26 for the corresponding arsenoxides. In the treatment of rabbit trypanosomiasis, the difference was not as marked but was again in favor of phenyl arsenoxides.

4. Various types of substituents have had fairly regular effects on the trypanocidal activity, toxicity, and thus on the chemotherapeutic index of phenyl arsenoxide.

(*a*) Nitro, chloro, methyl, amino, and hydroxyl groups had no significant effect on the activity: toxicity ratio.

(*b*) Acidic groups usually caused a striking decrease in trypanocidal action.

(*c*) Amide-substituted compounds, or those with a substituent containing a terminal acetamide group, were uniformly low in toxicity, and usually had a favorable activity: toxicity ratio, varying up to 7.4 times that of the unsubstituted compound. In the treatment of mouse trypanosomiasis the chemotherapeutic index $\left(\frac{LD_{50}}{CD_{50}}\right)$ of such compounds varied between 2.8 and 28.3, the corresponding index for the unsubstituted phenyl arsenoxide being less than 1.

(*d*) Substituting an amide hydrogen with $-CH_3$ or $-C_2H_5$ reacted unfavorably on the activity: toxicity ratio of the compound; but similar substitution with a group containing a terminal hydroxyl, acetamide, or nitrile linkage did not adversely affect the favorable effect of the amide group.

5. The most promising compound in the present series was the p-$(CH_2)_3COOH$ phenyl arsenoxide. This compound was an exception to the inhibitory effect of acidic-substituents on trypanocidal activity and had a molar activity in vitro 54 percent that of the unsub-

stituted compound. The LD_{50} value in mice on single intraperitoneal injection was 33.4 mg. per kg., the CD_{50} value in that species was 1.6 mg./kg., and the $\frac{LD_{50}}{CD_{50}}$ ratio of 20.5 was the third highest in the entire series. In rabbit trypanosomiasis treated by four consecutive daily injections, the LD_{50} value was 16 mg. per kg., the CD_{50} was 3.6 mg./kg., and the $\frac{LD_{50}}{CD_{50}}$ index was 4.4, the highest of all the compounds tested. The efficacy of this compound against strains of trypanosomes pathogenic for man is now under investigation.

REFERENCES

(*1*) Doak, G. O., Eagle, H., and Steinman, H. G.: The preparation of phenyl arsenoxides in relation to a projected study of their chemotherapeutic activity. I. Monosubstituted derivatives. J. Am. Chem. Soc., **62:** 168 (1940).

Doak, G. O., Steinman, H. G., and Eagle, H.: The preparation of phenyl arsenoxides. IV. Disubstituted compounds. J. Am. Chem. Soc., **63:** 99 (1941).

Doak, G. O., Eagle, H., and Steinman, H. G.: Arsine oxides of naphthalene and biphenyl. J. Am. Chem. Soc., **64:** 1064 (1942).

(*2*) Eagle, H., Hogan, R. B., Doak, G. O., and Steinman, H. G.: The toxicity, treponemicidal activity, and potential therapeutic utility of substituted phenyl arsenoxides. III. Monosubstituted compounds: Acids, esters, benzophenone, methylsulfone. J. Pharm. & Exp. Therap., **70:** 221 (1940).

————: The effect of multiple substituents on the toxicity and treponemicidal activity of phenyl arsenoxide, Ibid, **74:** 210 (1942).

————: Amide-substituted phenylarsine oxides and their derivatives: A group of compounds of possible utility in the treatment of syphilis. J. Am. Chem. Soc., **65:** 1236 (1943).

(*3*) Yorke, W., and Murgatroyd, F.: Studies in chemotherapy. III. The action in vitro of certain arsenical and antimonial compounds on *T. rhodesiense* and on atoxyl- and acriflavine-resistant strains of this parasite. Ann. Trop. Med. & Parasitol., **24:** 449 (1930).

(*4*) Eagle, H.: The toxicity, treponemicidal activity, and potential therapeutic utility of substituted phenyl arsenoxides. I. Methods of assay. J. Pharm. & Exp. Therap., **69:** 342 (1940).

(*5*) Reed, L. S., and Muench, H.: A simple method of estimating 50 percent end points. Am. J. Hyg., **27:** 493 (1938).

(*6*) Pearce, L., and Brown, W. H.: Experimental trypanosomiasis: its application in chemotherapeutic investigations. J. Exper. Med., **28:** 109 (1918).

(*7*) Kolmer, J. A., Kast, C., and Rule, A.: Spirocheticidal, trypanocidal, and mechanism of activity of organic arsenical compounds in vitro and in vivo in relation to therapeutic effectiveness. Am. J. Syph., **24:** 201 (1940).

(*8*) Ehrlich, P., and Hata, S.: The Experimental Chemotherapy of Spirilloses. Redman and Company, New York, 1911, p. 126.

(*9*) Probey, T. F., and McCoy, G. W.: Relation between trypanocidal and spirocheticidal activities of neoarsphenamine. Pub. Health Rep., **45:** 1716 (1930).

(*10*) Hawking, F.: Contributions on the mode of action of germanin (Bayer 205). Ann. Trop. Med. & Parasitol., **33:** 1 (1939).

(*11*) Yorke, W., Murgatroyd, F., and Hawking, F.: Studies in chemotherapy. IV. The action in vivo of certain arsenical and antimonial compounds and of Bayer 205 on *T. rhodesiense* and on atoxyl- and acriflavine-resistant strains of this parasite. Ann. Trop. Med. & Parasitol., **25:** 313 (1931).

————: V. Preliminary contribution on the nature of drug-resistance. Ibid, **25:** 351 (1931).

(*12*) Eagle, H., Hogan, R. B., Doak, G. O., and Steinman, H. G.: The toxicity and treponemicidal activity of amide-substituted phenyl arsenoxides and their derivatives. J. Pharm. & Exp. Therap. (in press).
(*13*) Gough, G. A. C., and King, H.: Trypanocidal action and chemical constitution. IX. Aromatic acids containing an 'amide 'group. J. Chem. Soc., 669 (1930).
(*14*) Hogan, R. B., and Eagle, H.: The pharmacologic basis for the widely varying toxicity of arsenicals. J. Pharm. & Exp. Therap., **80**: 93 (1944).

DEATHS DURING WEEK ENDED JUNE 3, 1944

[From the Weekly Mortality Index, issued by the Bureau of the Census, Department of Commerce]

	Week ended June 3, 1944	Corresponding week, 1943
Data for 93 large cities of the United States:		
Total deaths	8,436	9,005
Average for 3 prior years	8,496	
Total deaths, first 22 weeks of year	213,762	217,680
Deaths under 1 year of age	597	660
Average for 3 prior years	587	
Deaths under 1 year of age, first 22 weeks of year	13,778	15,133
Data from industrial insurance companies:		
Policies in force	66,588,800	65,548,808
Number of death claims	10,648	10,286
Deaths claims per 1,000 policies in force, annual rate	8.4	8.2
Death claims per 1,000 policies, first 22 weeks of year, annual rate	10.8	10.4

PREVALENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

REPORTS FROM STATES FOR WEEK ENDED JUNE 10, 1944

Summary

A total of 314 cases of meningococcus meningitis was reported for the current week, as compared with 274 last week, 382 for the corresponding week last year, and a 5-year (1939–43) median of 32. While the general trend is downward, the incidence fluctuates from week to week. Increases were reported currently in all of the 9 geographic areas except the West North Central, Mountain, and Pacific. Seven States, with reports of 19 to 36 cases each, reported an aggregate of 186 cases, as compared with 154 for the same States last week. The cumulative total since the week ended March 4, the last week in which the total exceeded the corresponding figure for last year, is 6,124, as compared with 7,344 for the same period last year.

A total of 41 cases of poliomyelitis was reported, as compared with 46 last week, 60 for the corresponding week last year, and a 5-year median of 54. The largest numbers of cases were reported in California (9), Louisiana (7), Kentucky (5), and New York (4). The total reported to date is 588, as compared with 659 for the same period last year and a 5-year median of 556.

Of a total of 104 cases of typhoid fever, as compared with 83 last week and a 5-year median of 124, 9 occurred in Oklahoma, 8 each in Texas and California, and 6 each in New York, Pennsylvania, Illinois, and Tennessee. The total for the year to date is 1,792, as compared with 1,425 last year and a 5-year median of 1,947.

Decreases were reported currently for the other 6 of the 9 common communicable diseases included in the following table, and only for scarlet fever is the current incidence above the corresponding 5-year median. Cumulative figures, however, for only diphtheria, smallpox, typhoid fever, and whooping cough are below the corresponding 5-year medians.

A total of 8,360 deaths was recorded for the week in 93 large cities of the United States, as compared with 8,436 last week and 8,445 for the 3-year (1941–43) average. The total for the year to date is 222,122, as compared with 226,890 for the same period last year.

Telegraphic morbidity reports from State health officers for the week ended June 10, 1944, and comparison with corresponding week of 1943 and 5-year median

In these tables a zero indicates a definite report, while leaders imply that, although none was reported, cases may have occurred.

Division and State	Diphtheria			Influenza			Measles			Meningitis, meningoccus		
	Week ended—		Median 1939–43	Week ended—		Median 1939–43	Week ended—		Median 1939–43	Week ended—		Median 1939–43
	June 10, 1944	June 12, 1943		June 10, 1944	June 12, 1943		June 10, 1944	June 12, [1943		June 10, 1944	June 12, 1943	
NEW ENGLAND												
Maine	0	0	0	----	2	----	139	191	147	1	5	1
New Hampshire	0	0	0	----	----	----	3	36	10	0	2	0
Vermont	0	0	0	----	----	----	14	293	105	0	0	0
Massachusetts	1	2	3	----	----	----	877	1, 532	1, 120	13	19	2
Rhode Island	0	0	0	16	----	----	6	81	106	2	6	0
Connecticut	1	2	0	----	----	1	425	342	342	1	8	0
MIDDLE ATLANTIC												
New York	6	8	17	(1)	2	7	1, 053	3, 784	1, 856	36	63	5
New Jersey	5	4	6	1	7	3	713	2, 172	1, 256	10	23	1
Pennsylvania	10	15	14	4	----	----	411	1, 007	715	26	27	6
EAST NORTH CENTRAL												
Ohio	2	7	8	2	2	7	162	315	315	36	15	1
Indiana	5	3	4	1	1	3	51	372	73	4	5	1
Illinois	1	23	23	9	5	11	345	1, 432	222	19	19	1
Michigan [2]	6	5	4	----	1	1	447	3, 352	832	21	28	1
Wisconsin	3	1	1	6	13	20	1, 431	2, 497	1, 219	9	3	0
WEST NORTH CENTRAL												
Minnesota	2	1	1	----	1	1	324	377	166	3	3	0
Iowa	2	5	3	----	----	----	105	97	167	0	0	0
Missouri	0	0	1	----	----	----	62	185	185	9	14	0
North Dakota	3	0	0	----	2	2	21	51	19	0	1	0
South Dakota	3	0	1	----	----	----	5	79	14	0	0	0
Nebraska	2	2	1	----	1	1	29	158	89	0	2	0
Kansas	3	2	2	----	----	2	133	287	287	3	7	0
SOUTH ATLANTIC												
Delaware	0	0	0	----	----	----	10	30	20	1	0	0
Maryland [2]	6	6	1	1	1	2	204	226	225	8	13	4
District of Columbia	1	0	0	----	1	----	60	89	89	2	7	1
Virginia	3	1	6	43	60	86	280	219	336	3	5	1
West Virginia	1	3	6	7	1	4	250	33	26	2	6	1
North Carolina	4	4	5	8	2	2	327	167	262	3	6	0
South Carolina	3	8	3	109	79	89	188	77	60	1	4	1
Georgia	3	1	3	----	17	17	37	97	97	2	4	0
Florida	2	0	5	1	4	4	111	18	71	7	3	0
EAST SOUTH CENTRAL												
Kentucky	1	2	4	----	2	3	42	63	63	9	1	1
Tennessee	2	7	3	10	11	16	72	103	103	10	10	1
Alabama	2	2	3	9	35	18	71	110	80	0	1	2
Mississippi [2]	4	2	3	----	----	----	----	----	----	9	3	0
WEST SOUTH CENTRAL												
Arkansas	4	7	4	12	6	11	163	55	55	1	0	0
Louisiana	8	1	3	4	----	2	21	17	18	6	0	1
Oklahoma	0	4	4	47	9	10	180	13	38	3	0	0
Texas	22	29	20	287	298	153	1, 172	228	437	20	9	2
MOUNTAIN												
Montana	0	0	0	4	4	1	43	110	110	0	3	0
Idaho	0	0	0	2	----	----	11	29	29	0	9	0
Wyoming	0	0	0	----	19	1	48	41	15	0	3	0
Colorado	8	13	8	12	56	18	103	151	151	2	2	0
New Mexico	1	0	0	----	6	2	58	3	12	0	1	0
Arizona	0	2	2	33	58	45	112	9	39	1	2	0
Utah [2]	0	0	0	1	----	----	71	112	112	1	3	0
Nevada	0	0	0	----	----	----	4	17	13	0	1	0
PACIFIC												
Washington	1	9	3	2	7	1	223	361	361	2	8	1
Oregon	0	2	1	3	10	9	111	105	72	0	6	2
California	23	17	16	42	42	49	3, 384	1, 163	1, 163	28	22	2
Total	154	200	200	676	765	765	14, 112	22, 286	14, 662	314	382	32
[3] 3 weeks	5, 075	5, 671	6, 016	333, 967	75, 514	147, 990	551, 741	466, 940	423, 156	11, 197	11, 104	1, 094

See footnotes at end of table.

Telegraphic morbidity reports from State health officers for the week ended June 10, 1944, and comparison with corresponding week of 1943 and 5-year median—Con.

Division and State	Poliomyelitis			Scarlet fever			Smallpox			Typhoid and paratyphoid fever [3]		
	Week ended—		Median 1939–43	Week ended—		Median 1939–43	Week ended—		Median 1939–43	Week ended—		Median 1939–43
	June 10, 1944	June 12, 1943		June 10, 1944	June 12, 1943		June 10, 1944	June 12, 1943		June 10, 1944	June 12, 1943	
NEW ENGLAND												
Maine	0	0	0	40	13	3	0	0	0	0	0	0
New Hampshire	0	1	0	3	9	1	0	0	0	0	0	0
Vermont	0	0	0	2	9	5	0	0	0	0	0	0
Massachusetts	0	0	0	251	360	166	0	0	0	3	4	4
Rhode Island	0	1	0	8	24	6	0	0	0	0	0	0
Connecticut	0	1	1	43	64	35	0	0	0	1	3	2
MIDDLE ATLANTIC												
New York	4	5	3	304	344	344	0	0	0	6	7	7
New Jersey	0	0	0	141	52	102	0	1	0	0	3	2
Pennsylvania	0	0	0	219	130	256	0	0	0	6	5	7
EAST NORTH CENTRAL												
Ohio	2	1	0	274	134	196	0	3	1	3	4	5
Indiana	0	1	0	59	54	42	0	1	1	0	5	1
Illinois	2	1	2	146	108	180	0	0	4	6	1	3
Michigan [2]	0	0	0	215	66	178	0	0	2	5	2	3
Wisconsin	0	0	0	151	237	93	1	0	1	1	1	1
WEST NORTH CENTRAL												
Minnesota	0	0	0	61	38	40	0	0	0	2	0	0
Iowa	0	0	0	127	12	14	0	0	0	1	0	0
Missouri	0	0	0	29	37	37	0	1	1	0	2	2
North Dakota	0	0	0	14	0	4	1	0	0	0	0	1
South Dakota	0	0	0	7	12	8	0	0	2	0	1	0
Nebraska	0	0	0	19	17	8	0	0	1	0	0	0
Kansas	0	0	0	34	24	27	0	0	0	1	2	1
SOUTH ATLANTIC												
Delaware	0	0	0	4	3	3	0	0	0	0	0	0
Maryland [2]	0	0	0	83	34	34	0	0	0	1	3	3
District of Columbia	0	0	0	32	6	6	0	0	0	1	0	0
Virginia	0	0	0	32	20	19	0	0	0	3	7	5
West Virginia	0	0	0	51	10	18	0	0	0	2	3	3
North Carolina	1	0	0	15	7	16	0	0	0	0	0	3
South Carolina	0	0	1	4	4	3	0	0	0	1	1	2
Georgia	1	0	0	9	11	5	0	0	0	2	15	13
Florida	1	0	1	3	0	1	0	0	0	2	7	5
EAST SOUTH CENTRAL												
Kentucky	5	2	1	23	10	35	1	0	0	5	4	4
Tennessee	1	0	0	28	10	26	0	0	2	6	3	3
Alabama	0	2	1	8	11	8	0	0	0	1	0	2
Mississippi [2]	3	0	0	6	3	3	0	0	0	0	2	2
WEST SOUTH CENTRAL												
Arkansas	0	1	0	2	5	2	0	1	1	5	7	7
Louisiana	7	0	1	2	2	4	0	1	0	4	5	5
Oklahoma	3	0	0	10	13	5	0	0	3	9	1	3
Texas	0	10	1	68	26	21	0	0	1	8	6	13
MOUNTAIN												
Montana	0	0	0	17	8	10	0	0	0	1	0	0
Idaho	0	0	0	25	66	2	0	0	0	2	0	0
Wyoming	0	0	0	25	17	2	0	0	0	0	0	0
Colorado	1	0	0	43	45	21	0	0	0	0	0	1
New Mexico	0	0	0	7	3	3	0	0	0	5	1	1
Arizona	0	3	0	24	11	6	0	0	0	2	0	1
Utah [2]	0	0	0	53	19	7	0	0	0	0	0	0
Nevada	0	1	0	0	1	0	0	0	0	0	0	0
PACIFIC												
Washington	1	3	0	129	20	20	0	0	0	1	0	0
Oregon	0	0	0	45	12	11	0	1	1	0	1	1
California	9	27	5	270	173	111	0	0	0	8	3	3
Total	41	60	54	3,165	2,294	2,294	3	9	42	104	109	124
23 weeks	588	659	556	135,274	87,636	87,636	251	553	1,037	1,792	1,425	1,947

See footnotes at end of table.

Telegraphic morbidity reports from State health officers for the week ended June 10, 1944, and comparison with corresponding week of 1943 and 5-year median—Con.

Division and State	Whooping cough			Week ended June 10, 1944								
	Week ended—		Median 1939–43	Anthrax	Dysentery			Encephalitis, infectious	Leprosy	Rocky Mt. spotted fever	Tularemia	Typhus fever
	June 10, 1944	June 12, 1943			Amebic	Bacillary	Unspecified					
NEW ENGLAND												
Maine	1	32	24	0	0	0	0	0	0	0	0	0
New Hampshire	0	15	6	0	0	0	0	0	0	0	0	0
Vermont	7	7	34	0	0	0	0	0	0	0	0	0
Massachusetts	56	132	162	0	0	0	0	1	0	0	0	0
Rhode Island	11	37	32	0	0	0	0	0	0	0	0	0
Connecticut	53	24	58	0	0	0	0	1	0	0	0	0
MIDDLE ATLANTIC												
New York	155	241	359	0	0	2	0	1	0	1	0	0
New Jersey	61	167	167	0	0	0	0	0	0	1	0	0
Pennsylvania	66	237	302	0	0	0	0	0	0	0	0	0
EAST NORTH CENTRAL												
Ohio	72	128	145	0	0	0	0	2	0	0	0	0
Indiana	16	57	50	0	0	0	0	0	0	0	0	0
Illinois	34	132	132	0	0	0	0	0	0	0	0	0
Michigan [2]	81	219	218	0	2	0	0	0	0	0	0	0
Wisconsin	48	246	125	0	0	0	0	0	0	0	0	0
WEST NORTH CENTRAL												
Minnesota	22	51	34	0	3	0	0	0	0	0	0	0
Iowa	4	23	26	0	0	0	0	0	0	0	0	0
Missouri	19	34	20	0	0	0	2	0	0	0	0	0
North Dakota	3	11	15	0	0	0	0	0	0	0	0	0
South Dakota	6	0	3	0	0	0	0	0	0	0	0	0
Nebraska	2	9	16	0	0	0	0	0	0	0	0	0
Kansas	30	75	55	0	0	0	0	0	0	0	0	0
SOUTH ATLANTIC												
Delaware		4	4	0	0	0	0	0	0	1	0	0
Maryland [2]	45	121	108	0	0	0	0	0	0	2	0	0
District of Columbia	1	41	24	0	0	0	0	0	0	0	0	0
Virginia	35	135	59	0	0	0	206	0	0	3	6	0
West Virginia	23	129	58	0	0	0	0	0	0	0	0	0
North Carolina	124	250	237	0	1	0	0	0	0	0	0	2
South Carolina	79	84	63	0	0	35	0	0	0	0	0	1
Georgia	30	90	25	0	1	27	1	0	0	0	1	20
Florida	19	21	10	0	0	166	0	4	0	0	0	9
EAST SOUTH CENTRAL												
Kentucky	76	55	55	0	0	3	0	0	0	0	0	0
Tennessee	21	69	54	0	0	0	3	1	0	0	1	0
Alabama	14	39	55	0	0	0	0	0	0	0	0	15
Mississippi [2]	0			0	0	0	0	0	0	0	6	4
WEST SOUTH CENTRAL												
Arkansas	26	47	42	0	0	3	0	0	0	0	2	0
Louisiana	3	7	7	0	1	2	0	0	0	0	0	12
Oklahoma	4	26	12	0	0	0	0	0	0	3	0	0
Texas	230	507	294	0	20	482	0	4	0	0	0	39
MOUNTAIN												
Montana	4	20	7	0	0	0	0	0	0	0	0	0
Idaho	9	0	2	0	0	0	0	0	0	1	0	0
Wyoming	9	0	6	0	0	0	0	0	0	4	1	0
Colorado	13	25	29	0	0	1	0	0	0	1	0	0
New Mexico	7	5	20	0	0	3	0	1	0	0	0	0
Arizona	8	23	23	0	1	1	54	0	0	0	0	0
Utah [2]	84	65	65	0	0	0	0	0	0	1	2	0
Nevada	0	2	2	0	0	0	0	0	0	0	0	0
PACIFIC												
Washington	15	60	65	0	0	0	0	0	0	0	0	0
Oregon	14	20	24	0	0	0	0	0	0	0	0	0
California	96	518	431	0	0	13	0	0	1	0	0	0
Total	1,736	4,240	3,778	0	29	738	266	15	1	18	19	102
23 weeks	41,503	93,259	90,631	17	591	6,740	1,909	256	14	90	257	1,100
23 weeks, 1943				31	769	4,890	1,237	258	11	108	415	1,061

[1] New York City only.
[2] Period ended earlier than Saturday.
[3] Including paratyphoid fever cases reported separately, as follows: Massachusetts 3, Illinois 1, Michigan 1, Georgia 1, Florida 1, Kentucky 1, Arkansas 1, Texas 1, California 1.

WEEKLY REPORTS FROM CITIES

City reports for week ended May 27, 1944

This table lists the reports from 90 cities of more than 10,000 population distributed throughout the United States, and represents a cross section of the current urban incidence of the diseases included in the table.

	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Poliomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
NEW ENGLAND												
Maine:												
Portland	0	0	-----	0	54	0	1	0	9	0	0	0
New Hampshire:												
Concord	0	0	-----	0	2	0	0	0	0	0	0	1
Vermont:												
Barre	0	0	-----	0	0	0	0	0	1	0	0	0
Massachusetts:												
Boston	2	0	-----	0	168	11	10	0	83	0	0	8
Fall River	0	0	-----	0	33	0	2	0	1	0	0	1
Springfield	0	0	-----	0	25	1	0	0	31	0	0	8
Worcester	0	0	-----	0	5	0	9	0	28	0	2	4
Rhode Island:												
Providence	0	0	-----	0	5	0	6	0	5	0	0	4
Connecticut:												
Bridgeport	0	0	-----	0	11	0	2	1	1	0	0	0
Hartford	0	0	-----	0	8	2	1	0	15	0	1	0
New Haven	0	0	-----	0	34	2	0	0	1	0	0	4
MIDDLE ATLANTIC												
New York:												
Buffalo	0	0	-----	-----	4	2	8	-----	7	0	0	0
New York	17	2	1	2	359	34	64	3	245	0	0	25
Rochester	0	0	-----	0	17	3	4	1	1	0	1	1
Syracuse	0	0	-----	0	4	0	3	0	4	0	0	9
New Jersey:												
Camden	1	0	-----	0	5	0	1	0	14	0	0	0
Newark	0	0	-----	0	146	1	5	0	24	0	0	3
Trenton	0	0	-----	0	2	0	0	0	7	0	0	0
Pennsylvania:												
Philadelphia	2	0	1	1	45	9	21	0	87	0	0	11
Pittsburgh	1	0	-----	0	4	3	11	0	23	0	0	5
Reading	0	0	-----	0	2	0	1	0	0	0	0	1
EAST NORTH CENTRAL												
Ohio:												
Cincinnati	1	0	-----	0	36	4	0	2	44	0	0	8
Cleveland	0	0	2	2	29	7	10	0	117	0	0	8
Columbus	1	0	-----	0	23	1	3	0	3	0	0	17
Indiana:												
Fort Wayne	0	0	-----	0	0	1	2	0	0	0	0	0
Indianapolis	3	0	-----	0	78	2	5	0	34	0	0	9
South Bend	0	0	-----	0	1	0	0	0	5	0	0	0
Terre Haute	0	0	-----	0	2	0	1	0	0	0	1	0
Illinois:												
Chicago	7	0	-----	0	142	14	14	0	112	0	0	17
Springfield	0	0	-----	0	45	0	1	0	2	0	1	0
Michigan:												
Detroit	2	0	-----	1	136	12	8	0	123	0	0	24
Flint	0	0	-----	0	3	0	1	0	6	0	0	2
Grand Rapids	0	0	-----	0	11	0	2	0	12	0	0	1
Wisconsin:												
Kenosha	0	0	-----	0	216	0	0	0	1	0	0	9
Milwaukee	0	0	-----	0	241	1	4	0	58	0	0	27
Racine	0	0	-----	0	92	0	0	0	1	0	0	3
Superior	0	0	-----	0	3	0	0	0	9	0	0	0
WEST NORTH CENTRAL												
Minnesota:												
Duluth	0	0	-----	0	157	0	1	0	8	0	0	-----
Minneapolis	0	0	-----	0	198	1	3	0	38	0	0	4
St. Paul	0	0	-----	0	57	1	2	0	14	0	0	2
Missouri:												
Kansas City	0	0	-----	0	62	1	6	0	8	0	0	2
St. Joseph	0	0	-----	0	-----	0	0	0	2	0	0	0
St. Louis	0	0	1	1	20	7	15	0	15	0	2	9
North Dakota:												
Fargo	0	0	-----	0	1	0	0	0	7	0	0	0
Nebraska:												
Omaha	1	0	-----	0	53	0	3	0	11	0	1	0
Kansas:												
Topeka	1	0	-----	0	35	0	2	0	0	0	0	0
Wichita	0	0	-----	0	14	0	2	0	6	0	0	3

City reports for week ended May 27, 1944—Continued

	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Poliomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
SOUTH ATLANTIC												
Delaware:												
Wilmington	0	0	.....	0	0	0	1	0	2	0	0	0
Maryland:												
Baltimore	8	0	2	2	138	1	6	0	39	0	0	43
Cumberland	0	1	.....	0	0	0	0	0	0	0	0	0
Frederick	0	0	.....	0	0	0	0	0	3	0	0	0
District of Columbia:												
Washington	0	0	.....	0	147	0	7	0	82	0	0	4
Virginia:												
Lynchburg	0	0	.....	0	0	0	0	0	2	0	0	0
Richmond	0	0	.....	0	10	2	1	0	3	0	0	0
Roanoke	1	0	.....	0	4	0	0	0	1	0	0	7
West Virginia:												
Charleston	0	0	.....	0	0	0	0	0	8	0	0	0
Wheeling	0	0	.....	0	0	0	3	0	0	0	0	0
North Carolina:												
Raleigh	0	0	.....	0	66	0	2	0	1	0	0	1
Wilmington	0	0	.....	0	17	0	1	0	0	0	0	8
Winston-Salem	0	0	.....	0	7	0	0	0	1	0	0	1
South Carolina:												
Charleston	0	0	.....	0	0	0	1	0	0	0	0	0
Georgia:												
Atlanta	0	0	5	0	9	0	4	0	15	0	0	0
Brunswick	0	0	.....	0	0	0	0	0	0	0	0	0
Savannah	0	0	.....	0	1	0	1	0	0	0	0	0
Florida:												
Tampa	1	0	3	0	1	1	1	0	1	0	3	0
EAST SOUTH CENTRAL												
Tennessee:												
Memphis	0	0	1	1	10	3	9	0	12	0	0	27
Nashville	0	0	.....	3	14	3	2	0	4	0	0	0
Aalabma:												
Birmingham	0	0	.....	0	7	1	1	0	2	0	0	0
Mobile	0	0	.....	0	2	1	2	0	0	0	0	0
WEST SOUTH CENTRAL												
Arkansas:												
Little Rock	0	0	1	1	7	0	2	0	0	0	0	0
Louisiana:												
New Orleans	0	0	2	0	20	3	6	0	1	0	1	1
Shreveport	0	0	.....	0	0	0	6	1	0	0	0	0
Texas:												
Dallas	0	0	.....	0	66	0	2	0	4	0	0	3
Galveston	0	0	.....	0	2	0	3	0	1	0	1	1
Houston	0	0	.....	0	1	1	4	0	2	0	0	1
San Antonio	1	0	1	2	4	0	2	0	2	0	0	1
MOUNTAIN												
Montana:												
Billings	0	0	.....	0	19	0	1	0	1	0	0	0
Great Falls	0	0	.....	0	5	0	0	0	1	0	0	0
Helena	0	0	.....	0	4	0	0	0	1	0	0	0
Missoula	0	0	.....	0	17	0	0	0	1	0	0	0
Idaho:												
Boise	0	0	.....	0	1	0	0	0	2	0	0	0
Colorado:												
Denver	1	0	2	0	48	2	3	1	14	0	0	11
Pueblo	0	0	.....	0	6	0	2	0	8	0	0	0
Utah:												
Salt Lake City	0	0	.....	0	15	0	2	0	21	0	0	7
PACIFIC												
Washington:												
Seattle	0	0	.....	0	50	1	3	0	23	0	0	2
Spokane	0	0	.....	0	32	0	0	0	11	0	0	0
Tacoma	0	0	.....	0	26	0	0	0	16	0	0	0
California:												
Los Angeles	6	.....	4	0	433	4	1	0	31	0	0	10
Sacramento	1	0	.....	0	75	0	0	0	10	0	1	0
San Francisco	0	0	.....	0	238	1	11	0	18	0	0	1
Total	58	3	26	16	4,090	144	323	9	1,567	0	13	359
Corresponding week, 1943	73	6	66	22	8,638	204	377	7	1,270	1	21	1,172
Average, 1939–43	67	.....	57	17	5,247	.....	313	.....	1,276	5	21	1,208

Dysentery, amebic.—Cases: New York, 2; Philadelphia, 1; Columbus, 2; Detroit, 1.
Dysentery, bacillary.—Cases: Providence, 1; New York, 4; Chicago, 2; Baltimore, 1; Houston, 2.
Dysentery, unspecified.—Cases: New Haven, 1; Baltimore, 2; Tampa, 1; San Antonio, 14.
Rocky Mountain spotted fever.—Cases: Indianapolis, 1; Denver, 1.
Typhus fever.—Cases: Fort Wayne, 3; Mobile, 1; San Antonio, 1.
Tularemia.—Cases: Houston, 1.

Rates (annual basis) per 100,000 population, by geograghic groups, for the 90 cities in the preceding table (estimated population, 1943, 34,519,500)

	Diphtheria case rates	Encephalitis, infectious, case rates	Influenza		Measles case rates	Meningitis, meningococcus, case rates	Pneumonia death rates	Poliomyelitis case rates	Scarlet fever case rates	Smallpox case rates	Typhoid and paratyphoid fever case rates	Whooping cough case rates
			Case rates	Death rates								
New England	5.2	0.0	0.0	0.0	898	41.6	80.7	2.6	455	0.0	7.8	78
Middle Atlantic	9.5	0.9	0.9	1.4	267	23.6	53.6	1.8	187	0.0	0.5	25
East North Central	8.5	0.0	1.2	1.8	645	25.6	31.1	1.2	321	0.0	1.2	76
West North Central	3.9	0.0	2.0	2.0	1,178	19.7	65.1	0.0	215	0.0	2.0	39
South Atlantic	16.3	1.6	16.3	3.3	653	6.5	45.7	0.0	258	0.0	4.9	104
East South Central	0.0	0.0	5.8	23.3	192	46.6	81.6	0.0	105	0.0	0.0	157
West South Central	2.8	0.0	11.4	8.5	284	11.4	71.0	0.0	28	0.0	5.7	20
Mountain	7.9	0.0	15.8	0.0	911	15.8	63.4	2.8	388	0.0	0.0	143
Pacific	11.5	0.0	6.6	0.0	1,409	9.9	24.7	7.9	180	0.0	1.6	21
Total	8.8	0.5	3.9	2.4	620	21.8	48.9	1.4	237	0.0	1.9	54

PLAGUE INFECTIONS IN QUAY AND UNION COUNTIES, N. MEX.

Plague infection has been reported proved in a pool of 60 fleas from 2 wood rats, *Neotoma albigula*, collected on May 10, 1944, 20 miles east of Tucumcari, on U. S. Highway No. 66, in Quay County, N. Mex., and in a pool of 22 fleas from grasshopper mice, *Onychomys leucogaster*, collected on May 11 at locations 18–23 miles south of Clayton, on Highway No. 18, in Union County, N. Mex.

Tularemia.—Cases: Houston, 1.

FOREIGN REPORTS

CANADA

Provinces—Communicable diseases—Week ended May 13, 1944.—During the week ended May 13, 1944, cases of certain communicable diseases were reported by the Dominion Bureau of Statistics of Canada as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Chickenpox		6	2	160	337	39	21	108	175	848
Diphtheria		6	3	20	1	1	1		1	33
German measles		19		200	235	16	52	20	58	600
Influenza			1		16		7		5	29
Measles		8	9	900	723	380	67	122	33	2,242
Meningitis, meningococcus				1	1				1	3
Mumps	1	21		175	275	17	12	108	38	647
Scarlet fever		24	9	74	275	62	13	95	80	632
Tuberculosis		16		249	85	27	13	21	27	438
Typhoid and paratyphoid fever				12	1	4	1			18
Undulant fever				2	1					3
Whooping cough		27		40	34	2	1	18	22	144

CUBA

Provinces—Notifiable diseases—Correction.—Reports of certain notifiable diseases by Provinces in Cuba as published on page 565 of the PUBLIC HEALTH REPORTS of the issue of April 28, 1944, should be corrected as follows: Tularemia should be undulant fever, Oriente 1, total 1; yaws should be Oriente 5, total 5. It is believed that no cases of tularemia have occurred in Cuba.

FINLAND

Notifiable diseases—March 1944.—During the month of March 1944, cases of certain notifiable diseases were reported in Finland as follows:

Disease	Cases	Disease	Cases
Actinomycosis	1	Paratyphoid fever	191
Cerebrospinal meningitis	27	Pneumonia (all forms)	2,088
Chickenpox	607	Poliomyelitis	12
Conjunctivitis	17	Puerperal fever	35
Diphtheria	1,270	Rheumatic fever	288
Dysentery	6	Scabies	2,421
Gastroenteritis	1,603	Scarlet fever	924
Gonorrhea	516	Syphilis	350
Hepatitis, epidemic	523	Tetanus	1
Influenza	2,482	Typhoid fever	34
Laryngitis	53	Undulant fever	1
Measles	3,962	Vincent's angina	9
Mumps	534	Whooping cough	920

JAMAICA

Notifiable diseases—4 weeks ended May 6, 1944.—During the 4 weeks ended May 6, 1944, cases of certain notifiable diseases were reported in Kingston, Jamaica, and in the island outside of Kingston, as follows:

Disease	Kingston	Other localities	Disease	Kingston	Other localities
Cerebrospinal meningitis		1	Leprosy		2
Chickenpox	10	51	Tuberculosis	40	84
Diphtheria	3	3	Typhoid fever	8	56
Dysentery		2	Typhus fever	10	2
Erysipelas		1			

NEW ZEALAND

Notifiable diseases—4 weeks ended April 22, 1944.—During the 4 weeks ended April 22, 1944, certain notifiable diseases were reported in New Zealand as follows:

Disease	Cases	Deaths	Disease	Cases	Deaths
Actinomycosis	1		Lethargic encephalitis	1	1
Cerebrospinal meningitis	10	2	Poliomyelitis	8	
Diphtheria	87	4	Puerperal fever	8	1
Dysentery (bacillary)	19		Scarlet fever	485	1
Erysipelas	25	1	Trachoma	2	
Food poisoning	2		Tuberculosis (all forms)	120	49
Influenza	2	1	Typhoid fever	7	1
Lead poisoning	1		Undulant fever	4	

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

NOTE.—Except in cases of unusual incidence, only those places are included which had not previously reported any of the above-mentioned diseases, except yellow fever, during the current year. All reports of yellow fever are published currently.

A table showing the accumulated figures for these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

(Few reports are available from the invaded countries of Europe and other nations in war zones.)

Cholera

India—Calcutta.—During the week ended May 13, 1944, 100 deaths from cholera were reported in Calcutta, India.

Plague

Egypt.—Plague has been reported in Egypt as follows: Ismailiya—week ended May 26, 1944, 34 cases with 20 deaths including 13 cases and 7 deaths in the southern areas; Port Said—week ended May 13, 1944, 2 cases.

French West Africa—Dakar District—Island of Goree.—For the week ended May 6, 1944, 1 case of plague was reported on the Island of Goree, Dakar District, French West Africa.

Indochina.—Plague has been reported in Indochina as follows: April 21–30, 1944, Annam, 1 case, Cochinchina, 3 cases; May 1–10, 1944, Annam, 6 cases.

Madagascar.—For the period March 11–20, 1944, 4 cases of plague were reported in Madagascar.

Smallpox

Cameroon (French).—For the period April 1–20, 1944, 143 cases of smallpox were reported in French Cameroon.

India.—Smallpox has been reported in India as follows: Bombay—week ended April 29, 1944, 78 cases, 25 deaths; week ended May 6, 1944, 74 cases, 27 deaths; Calcutta—week ended May 13, 1944, 267 deaths.

Nigeria.—For the week ended April 29, 1944, 124 cases of smallpox with 21 deaths were reported in Nigeria.

Turkey.—For the month of March 1944, 851 cases of smallpox were reported in Turkey.

Typhus Fever

Iraq.—Typhus fever has been reported in Iraq as follows: Week ended April 22, 1944, 27 cases, 1 death; week ended April 29, 1944, 26 cases, 4 deaths.

Belgium—Namur Province—Tamines.—For the week ended May 6, 1944, 1 case of typhus fever was reported in Tamines, Namur Province, Belgium.

Hungary.—During the week ended May 13, 1944, 158 cases of typhus fever (including 70 cases in Subcarpathia) were reported in Hungary.

Irish Free State—Roscommon County—Castlerea.—For the week ended May 20, 1944, 1 case of typhus fever was reported in Castlerea, Roscommon County, Irish Free State.

Palestine.—For the month of April 1944, 76 cases of typhus fever with 6 deaths were reported in Palestine.

Tunisia.—Typhus fever has been reported in Tunisia as follows: April 11–20, 1944, 30 cases; April 21–30, 1944, 37 cases.

Yellow Fever

Belgian Congo—Stanleyville Province—Bondo.—For the week ended June 3, 1944, 1 death from yellow fever was reported in Bondo, Stanleyville Province, Belgian Congo.

COURT DECISIONS ON PUBLIC HEALTH

Municipality furnishing water supply in private capacity held to be employer subject to statute designed to protect employees from occupational disease.—(Missouri Supreme Court, Division No. 1; *Lockhart* v. *Kansas City*, 175 S.W.2d 814; decided December 6, 1943.) The plaintiff brought an action for damages on account of personal injuries and disease, claimed to have been caused by conditions under which he worked as janitor in the chemical building at the defendant city's water purification plant. It was alleged that substances prepared and used by the defendant caused deleterious and poisonous dust in the building where the plaintiff worked so that such dust was inhaled by him in dangerous quantities and caused permanent incapacitating injuries and disease. In addition to charging common law negligence the plaintiff also charged violation of certain health and safety statutes. The city contended that these statutes had no application to a municipal corporation and whether or not they were so applicable was the question presented for decision to the Supreme Court of Missouri.

This court first pointed out that cities had statutory authority to erect, maintain, and operate waterworks and other specified plants and that it had long been settled that such plants, when operated to supply services to individuals, were operated by a city in its private corporate capacity. With respect to the statutory provisions relied on by the plaintiff, one of the sections involved (section 10211 of the Missouri Revised Statutes) required "every employer of labor in this state" carrying on work which might produce occupational disease to adopt means to prevent same, while another section (10225) provided that "in this article, unless the context otherwise requires, 'employer' includes persons, partnerships, and corporations." The court said that it was apparent that the purpose was to protect the health of persons employed in processes likely to cause occupational diseases by requiring certain safeguards and preventive measures for the protection of the employees. The act was a recognition by the legislature that many chemical processes of modern industry were likely to cause occupational diseases unless such preventive measures were taken and that common law standards of care were inadequate to meet the situation. "Certainly such diseases would be just as harmful, both to the injured individual and the public, regardless of whether caused by conditions existing in a plant operated by a private or a municipal employer." Also, in the court's view, the law was not one to classify employers but one setting up new standards of care for carrying on certain essential industrial and manufacturing processes. The basis of its application was dependent upon the type of process in which the employee was engaged rather than who or what the employer

was. Because of the plain purpose of the law to protect the health of employees engaged in the specified dangerous processes, the appellate court's conclusion was that the act established standards of care for such work to replace inadequate common law standards, that such standards had to be observed by "every employer" carrying on work to which the act applied, and that no employer was excluded from the act who was liable for failure to observe common law standards of care. A municipality engaged in furnishing public utility services in its private corporate capacity was, therefore, held to be subject to the statute.

Certified copy of death certificate as prima facie evidence.—(Georgia Court of Appeals, Division No. 2; *Bituminous Casualty Corporation et al.* v. *Elliott*, 28 S.E.2d 392; decided November 26, 1943; rehearing denied December 14, 1943.) Section 88–1214 of the Georgia code provided that one of the items to be contained on a death certificate was the following: "Certification as to medical attendance on decedent, fact and time of death, time last seen alive, and cause of death, with contributory (secondary) cause or complication, if any, and duration of each, and whether attributed to dangerous or insanitary conditions of employment; signature and address of physician or official making the medical certificate." The said section also provided that "The personal and statistical particulars (items 1 to 13) shall be authenticated by the signature of the informant, who may be any competent person acquainted with the facts," and that "The medical certificate shall be made and signed by the physician, if there was any, last in attendance on the deceased, who shall specify the time in attendance, the time he last saw the deceased alive, and the hour of the day at which the death occurred." Such physician was also required to "further state the cause of the death, so as to show the course of the disease or sequence of causes resulting in the death, giving first the name of the disease causing death (primary cause) and the contributory (secondary) cause, if any, and the duration of each." Section 88–1215 of the code provided for the making of a certificate where the death occurred without medical attendance, while by section 88–1212 a certified copy of the record of a death registered under the provisions of the vital statistics law was made "prima facie evidence in all courts and places of the facts therein stated."

In a proceeding under the State workmen's compensation act, wherein a widow claimed compensation for the death of her husband, it appeared that the physician who signed the death certificate last saw the deceased alive almost 60 days before his death. The death certificate did not purport to be under section 88–1215 but under section 88–1214, and the Georgia Court of Appeals took the view that

the certificate should have been made in accordance with section 88–1215 or by the physician in attendance on the deceased, if there was one. It followed, according to the court, that as a matter of law the certificate introduced was not prima facie evidence of the facts therein stated relative to the primary and secondary causes of the death of the employee.

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